Remarks

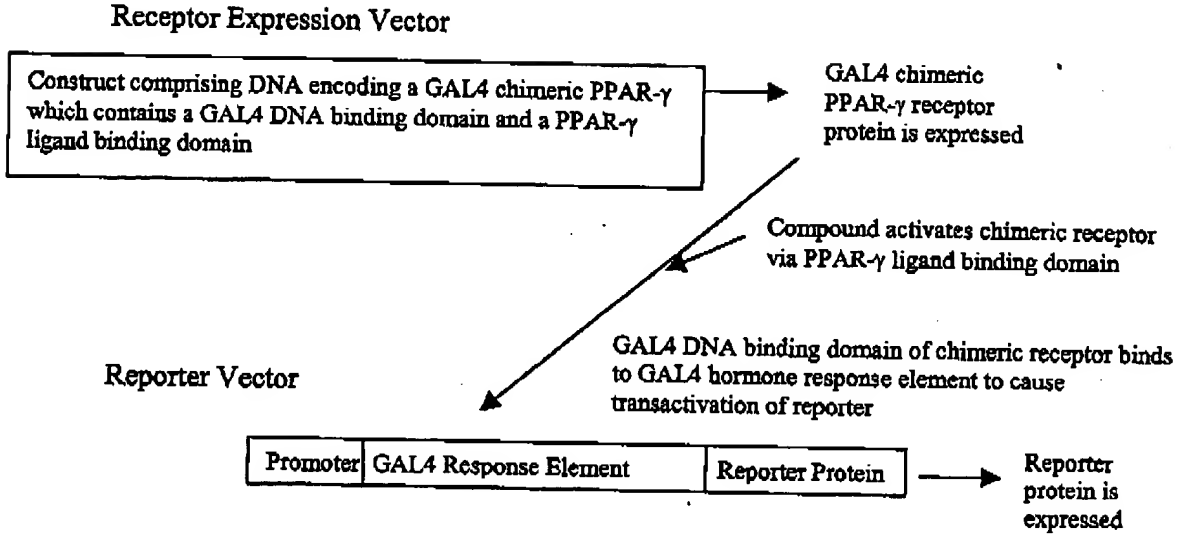
Courtesies extended to Applicants' representative in the personal interview held on November 21, 2002, are acknowledged with appreciation.

In accordance with the present invention, there are provided methods for testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ). Invention methods comprise assaying for changes in the level of reporter protein present as a result of contacting cells containing PPAR-γ (either endogenous to the host cell or introduced recombinantly) and a reporter vector with the compound of interest. Compounds identified employing invention methods are useful in the treatment of pathological conditions such as diabetes.

Claims 16-20 and 27-35 were pending before this communication. By this communication, new claims 36-45 have been presented, and claims 16, 20, 27-29 and 33-35 have been amended to define Applicants' invention with greater particularity. These amendments add no new matter and are fully supported by the specification and the original claims. Attached hereto is a marked-up version of the changes made to the claims, labeled APPENDIX A.

Accordingly, claims 16-20, and 27-45 are currently pending. For the Examiner's convenience, a clean copy of all claims is provided in APPENDIX B.

As discussed during the personal interview, the newly presented claims are specifically directed to the situation where the PPAR-γ component used in the functional bioassay is a GAL4 chimeric PPAR-γ receptor, as shown in Example 3 (see specification at pages 23-24). Claims 36-45, like the previously pending claims, are directed to methods of testing compounds in a functional bioassay system. The methods of claims 36-45 require testing compounds using cells that contain (i) a GAL4 chimeric PPAR-γ, and (ii) a reporter vector. A schematic of this embodiment of the present invention is provided below.



Applicants respectfully submit that claims 36-45 satisfy all requirements of 35 U.S.C. § 112, first paragraph. The scope of the claims is commensurate with the disclosure provided by the specification. Accordingly, any prior rejection of claims 16-20, 27 and 28 under 35 U.S.C. § 112, first paragraph, is not applicable to newly presented claims 36-45.

Conclusion

In view of the above amendments and remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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Enclosures: Appendices A and B

APPENDIX A – ALTERED CLAIMS
VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 16, 20, 27-29 and 33-35 have been amended as follows:

16. (Thrice amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding PPAR-γ, and

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein [~~detected~~] when said cells are contacted with said compound, relative to the level of the reporter protein [~~detected~~] when said cells are not contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

20. (Twice amended) A method according to claim 16 wherein said compound is a putative antagonist for said PPAR-γ [~~peroxisome proliferator-activated receptor-gamma~~], and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said PPAR-γ [~~peroxisome proliferator-activated receptor-gamma~~],

wherein a decrease in the level of the reporter protein [~~detected~~] when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein [~~detected~~] when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

27. (Twice amended) A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one PPAR-γ agonist,

wherein an increase or decrease in the level of the reporter protein [~~detected~~] when cells are contacted with said compound and said agonist, relative to the level of the reporter protein [~~detected~~] when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

28. (Twice amended) A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one PPAR-γ antagonist,

wherein an increase or decrease in the level of the reporter protein [~~detected~~] when cells are contacted with said compound and said antagonist, relative to the level of the reporter protein [~~detected~~] when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

29. (Amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said cells express native PPAR-γ, and

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein **[~~detected~~]** when said cells are contacted with said compound, relative to the level of the reporter protein **[~~detected~~]** when said cells are not contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

33. (Amended) A method according to claim 29, wherein said compound is a putative antagonist for said **PPAR-γ** **[~~peroxisome proliferator-activated receptor-gamma~~]**, and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said **PPAR-γ** **[~~peroxisome proliferator-activated receptor-gamma~~]**,

wherein a decrease in the level of the reporter protein **[~~detected~~]** when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein **[~~detected~~]** when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

34. (Amended) A method according to claim 29, wherein said contacting is carried out in the further presence of at least one PPAR-γ agonist,

wherein an increase or decrease in the level of the reporter protein [~~detected~~] when cells are contacted with said compound and said agonist, relative to the level of the reporter protein [~~detected~~] when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

35. (Amended) A method according to claim 29, wherein said contacting is carried out in the further presence of at least one PPAR-γ antagonist,

wherein an increase or decrease in the level of the reporter protein [~~detected~~] when cells are contacted with said compound and said antagonist, relative to the level of the reporter protein [~~detected~~] when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

APPENDIX B – CURRENTLY PENDING CLAIMS

16. (Thrice amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding PPAR-γ, and

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein when said cells are contacted with said compound, relative to the level of the reporter protein when said cells are not contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

17. (Reiterated) A method according to Claim 16 wherein said hormone response element is a direct repeat of two or more half sites separated by a spacer of one nucleotide, wherein said spacer can be A, C, G or T, wherein each half site comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-; and

wherein said response element is optionally preceded by N_x, wherein x falls in the range of 0 up to 5.

18. (Previously amended) A method according to claim 17 wherein said response element has at least one copy of the minimal sequence:

AGGACA A AGGTCA (SEQ. ID NO. 5),

wherein said minimal sequence is optionally flanked by additional residues.

19. (Previously amended) A method according to claim 17 wherein said response element has at least one copy of the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ. ID NO. 6).

20. (Twice amended) A method according to claim 16 wherein said compound is a putative antagonist for said PPAR-γ, and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said PPAR-γ,

wherein a decrease in the level of the reporter protein when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

27. (Twice amended) A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one PPAR-γ agonist,

wherein an increase or decrease in the level of the reporter protein when cells are contacted with said compound and said agonist, relative to the level of the reporter protein when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

28. (Twice amended) A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one PPAR-γ antagonist,

wherein an increase or decrease in the level of the reporter protein when cells are contacted with said compound and said antagonist, relative to the level of the reporter protein when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

29. (Amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said cells express native PPAR-γ, and

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein when said cells are contacted with said compound, relative to the level of the reporter protein when said cells are not

contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

30. (Reiterated) A method according to claim 29, wherein said hormone response element is a direct repeat of two or more half sites separated by a spacer of one nucleotide, wherein said spacer can be A, C, G or T, wherein each half site comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-; and

wherein said response element is optionally preceded by N_x, wherein x falls in the range of 0 up to 5.

31. (Reiterated) A method according to claim 30, wherein said response element has at least one copy of the minimal sequence:

AGGACA A AGGTCA (SEQ. ID NO. 5),

wherein said minimal sequence is optionally flanked by additional residues.

32. (Reiterated) A method according to claim 30, wherein said response element has at least one copy of the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ. ID NO. 6).

33. (Amended) A method according to claim 29, wherein said compound is a putative antagonist for said PPAR-γ, and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said PPAR-γ,

wherein a decrease in the level of the reporter protein when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

34. (Amended) A method according to claim 29, wherein said contacting is carried out in the further presence of at least one PPAR-γ agonist,

wherein an increase or decrease in the level of the reporter protein when cells are contacted with said compound and said agonist, relative to the level of the reporter protein when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

35. (Amended) A method according to claim 29, wherein said contacting is carried out in the further presence of at least one PPAR-γ antagonist,

wherein an increase or decrease in the level of the reporter protein when cells are contacted with said compound and said antagonist, relative to the level of the reporter protein when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

36. (New) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing a GAL4 chimeric PPAR-γ receptor and a reporter vector with said compound;

wherein said GAL4 chimeric PPAR-γ receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding at least the ligand binding domain of a PPAR-γ and a DNA segment encoding a GAL4 DNA binding domain, and

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a GAL4 response element capable of being bound by said GAL4 DNA binding domain, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said GAL4 response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein when said cells are contacted with said compound, relative to the level of the reporter protein when said cells are not contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

37. (New) A method according to claim 36, wherein the DNA segment encoding said GAL4 DNA binding domain is introduced at the amino terminus of the DNA segment encoding said ligand binding domain of a PPAR-γ.

38. (New) A method according to claim 36, wherein the DNA segment encoding said GAL4 DNA binding domain is introduced at the carboxy terminus of the DNA segment encoding said ligand binding domain of a PPAR-γ.

39. (New) A method according to claim 36, wherein the DNA segment encoding the native DNA binding domain of PPAR-γ is substituted with the DNA segment encoding said GAL4 DNA binding domain.

40. (New) A method according to claim 36, wherein the DNA segment encoding said GAL4 DNA binding domain encodes amino acid residues 1-147 of the GAL4 protein.

41. (New) A method according to claim 36, wherein the DNA segment encoding said GAL4 DNA binding domain encodes amino acid residues 1-90 of the GAL4 protein.

42. (New) A method according to claim 36, wherein the DNA segment encoding said GAL4 DNA binding domain encodes amino acid residues 1-74 of the GAL4 protein.

43. (New) A method according to claim 36, wherein said compound is a putative antagonist for said PPAR-γ, and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said PPAR-γ,

wherein a decrease in the level of the reporter protein when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

44. (New) A method according to Claim 36, wherein said contacting is carried out in the further presence of at least one PPAR-γ agonist,

wherein an increase or decrease in the level of the reporter protein when cells are contacted with said compound and said agonist, relative to the level of the reporter protein when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

45. (New) A method according to Claim 36, wherein said contacting is carried out in the further presence of at least one PPAR-γ antagonist,

wherein an increase or decrease in the level of the reporter protein when cells are contacted with said compound and said antagonist, relative to the level of the reporter protein when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.